The RNA Polymerase II Elongation Complex

FACTOR-DEPENDENT TRANSCRIPTION ELONGATION INVOLVES NASCENT RNA CLEAVAGE*

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Regulation of transcription elongation is an important mechanism in controlling eukaryotic gene expression. SII is an RNA polymerase II-binding protein that stimulates transcription elongation and also activates nascent transcript cleavage by RNA polymerase II in elongation complexes in vitro (Reines, D. (1992) J. Biol. Chem. 267, 3795–3800). Here we show that SII-dependent in vitro transcription through an arrest site in a human gene is preceded by nascent transcript cleavage. RNA cleavage appeared to be an obligatory step in the SII activation process. Recombinant SII activated cleavage while a truncated derivative lacking polymerase binding activity did not. Cleavage was not restricted to an elongation complex arrested at this particular site, showing that nascent RNA hydrolysis is a general property of RNA polymerase II elongation complexes. These data support a model whereby SII stimulates elongation via a ribonuclease activity of the elongation complex.

Expression of many genes in eukaryotes requires transcription of extremely large stretches of DNA by RNA polymerase II. During this transcription, RNA polymerase II encounters signals which block full-length primary-transcription synthesis (reviewed by Spencer and Groudine, 1990; Kerppola and Kane, 1991). Transcription in vitro using partially purified or defined components has resulted in the identification and characterization of RNA polymerase II transcription arrest sites (Spencer and Groudine, 1990). In some cases, arrest occurs at a bend in the helical axis of template DNA (Kerppola and Kane, 1990). Examples include sites within the first intron of the human c-myc gene, the first intron of the human histone H3.3 gene, and an early transcription unit of SV40. It has been suggested that at least part of the information that halts transcription resides in a bend-inducing structural element and that the bent template may provide a steric block to translocation of the enzyme (Kerppola and Kane, 1990).

In vitro, the RNA polymerase II elongation complex is extremely stable, as might be expected in order for RNA polymerase II to transcribe very large genes. However, since transcription can become efficiently blocked at numerous sites both in vitro and in vivo, it is important to understand how an arrested transcription complex can become reactivated for transcription. The transcription of megabase-sized genes and the regulation of growth controlling genes probably relies upon accessory transcription elongation factors to govern the efficiency of primary transcript synthesis.

Factors that influence transcription elongation by RNA polymerase II have been identified (Sekimizu et al., 1976; Spindler, 1979; Sawadogo et al., 1980; Reinberg and Roeder, 1987; Price et al., 1987; Bengal et al., 1991; Chafin et al., 1991). SII is an RNA polymerase II protein that is elongation factor SII, which is not required for initiation. SII has been purified from cultured mouse cells (Sekimizu et al., 1976), yeast (Sawadogo et al., 1980), Drosophila (Price et al., 1987), HeLa cells (Reinberg and Roeder, 1987; Reines et al., 1989), calf thymus (Rappaport et al., 1987), and bovine brain (Reines, 1992). cDNAs have been isolated for the mouse (Hirashima et al., 1988), yeast (Hubert et al., 1983; Clark et al., 1991), Drosophila (Marshall et al., 1990), and human (Yoo et al., 1991) factors.

SII is a 35–38 kDa polypeptide that allows RNA polymerase II to readthrough a pause in the adenovirus major late transcription unit (Reinberg and Roeder, 1987). This pause is important in regulating the viral infectious cycle (Maderious and Chen-Kiang, 1984, Mok et al., 1984). SII also enables RNA polymerase II to readthrough a transcription arrest signal (called site Ia) in the first intron of a human histone gene (Reines et al., 1989; SivaRaman et al., 1990). This site may serve to regulate the gene’s expression pattern during the cell cycle. The means by which SII activates transcription elongation is unknown.

Two properties of SII may provide clues to its mechanism of stimulating elongation. SII binds RNA polymerase II (Sawadogo et al., 1980; Horikoshi et al., 1984; Reinberg and Roeder, 1987; Rappaport et al., 1988). SII can also activate a previously unrecognized ribonuclease activity of elongation complex-associated RNA polymerase II (Reines, 1992). In the presence of SII, nascent transcript cleavage shortens the substrate RNA from its 3'-end by a few nucleotides, although more extensive digestion has also been observed.

To determine whether SII’s RNA polymerase binding activity and its nuclease activating activity are involved in efficient transcription through intragenic regions, we have examined a discrete population of purified elongation complexes arrested at a well defined site within a human histone gene. We show that transcript cleavage precedes SII-dependent transcript elongation and that an elongation complex containing a shortened RNA is an intermediate in SII activation of RNA chain elongation. A truncated form of SII that is incapable of binding RNA polymerase II cannot activate RNA shortening. These findings support a model in which:

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SII activation of a RNA polymerase II-associated nuclease is integral to the transcriptional readthrough process. Furthermore, transcript cleavage is not restricted to elongation complexes arrested at this SII-sensitive site in the human histone gene; therefore, nascent RNA cleavage activity is a general property of the RNA polymerase II elongation complex.

MATERIALS AND METHODS

Proteins and Reagents—RNA polymerase II and SII-free transcription initiation factors were purified from rat liver as described (Conaway et al., 1987; Reines, 1991a). Yeast inorganic pyrophosphatase was obtained from Sigma. Acetylated bovine serum albumin, T7 RNA polymerase, and EcoRI were supplied by Promega Biotech. RNAAgd, NdeI, PvuII, and unlabeled, fast protein liquid chromatography-purified nucleotides were purchased from Pharmacia LKB Biotechnology Inc. [α-32P]CTP was purchased from Amer sham Corp. Protein concentration was determined with protein assay reagent (Bio-Rad) according to the supplier's directions using bovine serum albumin (Sigma, Fraction V) as a standard. DNA dye reagent (Bio-Rad) according to the supplier's directions using bromophenol blue in TBE (89 mM Tris, 89 mM boric acid, pH 8.0, 0.3 M NaCl, 2% [w/v] SDS), and RNA was isolated as described (Reines, 1992).

RESULTS

Recombinant SII Synthesized in Vitro Stimulates the Ribonuclease Function of an RNA Polymerase II Elongation Complex—To confirm that the addition of SII is sufficient to activate the ribonuclease activity of RNA polymerase II, and to test whether RNA polymerase II-binding by SII is required for this activation, SII and a truncated form of SII (ΔSII) were prepared from a mouse cDNA clone (Hirashima et al., 1988) by in vitro transcription and translation in a wheat germ extract. The truncated derivative lacked its carboxyterminal 107 amino acids, in which residues important for RNA polymerase II binding reside (Horikoshi et al., 1990; Agarwal et al., 1991). [35S]SII and [35S]ΔSII were separated from the bulk of the wheat germ translation extract proteins by chromatography on phosphocellulose. These in vitro synthesized proteins were analyzed for RNA polymerase II binding activity by testing their ability to coimmunoprecipitate RNA polymerase II. A monoclonal antibody against the carbonyl-terminal domain (CTD) of the large subunit of RNA polymerase II (8WG16; Thompson et al., 1989) was used to isolate CTD-containing enzyme from a preparation of partially purified rat liver RNA polymerase II. The immunoprecipitate containing 8WG16 and RNA polymerase II was resuspended with 32P-labeled SII or ΔSII (Fig. 1A). Efficient precipitation of the full-length protein required both the antiRNA polymerase II antibody (Fig. 1A, lanes 3–8) and RNA polymerase II.ΔSII was not significantly precipitable above background when present at the same concentration at which SII was efficiently precipitated (Fig. 1A, lanes 7–10). The relatively high background of precipitable SII was reproducible, detected when protein A-Sepharose was the immunosorbent, and could not be reduced after further washing of the immunoprecipitate. These data independently confirm previous findings in which the region of SII important for RNA polymerase II binding was identified (Horikoshi et al., 1990; Agarwal et al., 1991). Furthermore, binding of 8WG16 to the CTD of RNA polymerase II did not prevent the subsequent binding of SII to the enzyme.

Recombinant SII proteins were also tested for their ability to activate SII-dependent nascent transcript cleavage (Fig. 1B). We have recently shown that RNA chains contained in

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3 The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.

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active elongation complexes can be cleaved after exposure to partially purified bovine brain SII (Reines, 1992). Only RNA in an elongation complex and not free RNA was cleaved. The nuclease activity was inhibited by α-amanitin (1 μg/ml). The simplest interpretation of those findings was that RNA polymerase II itself contains this catalytic function.

Elongation complexes containing [32P]RNA were assembled by transcription in vitro of a region of the human H3.3 histone gene that arrests transcription with high efficiency (Reines et al., 1987, 1989). Arrested elongation complexes were isolated and washed free of nucleotides by immunoprecipitation using an anti-RNA monoclonal antibody (Reines, 1991a, 1991b). Only full-length recombinant SII (Fig. 1B, lanes 2 and 3) was capable of activating nascent transcript cleavage. Truncated SII (Fig. 1B, lanes 4 and 5) was ineffective in stimulating transcript shortening, even when present at a molar concentration 10-fold higher than that of the full-length protein. Wheat germ translation extracts alone, or extracts programmed with control RNA, were also ineffective in activating the nuclease activity. These data demonstrated that SII was responsible for activating the nuclease activity of the RNA polymerase II elongation complex and that it need not be synthesized in mammalian cells to acquire its function as an activator of RNA cleavage. It also provides evidence that SII's polymerase binding activity is important for the activation of RNA cleavage.

Transcript Shortening Precedes SII-mediated Elongation Through Site Ia—RNA cleavage by the elongation complex is rapid; all precursor RNA is shortened in less than 1 min at 28 °C (Reines, 1992). In the absence of nucleotides, the half-life of a shortened RNA can be as long as 1 h (Reines, 1992). It was important then, to determine if the nuclease activity was operative under conditions where SII stimulates transcriptional readthrough, i.e., in the presence of SII and nucleotides. Since in this experiment we did not wish to deplete the complexes of nucleotides, they were not immunoprecipitated with anti-RNA antibody. A time course of SII-dependent elongation through this region showed that shortened transcripts were detected after 20 s (Fig. 2). The half-life of the smallest detectable intermediate was less than 25 s. Compared to the more extensively cleaved RNAs seen when no nucleotides were present (Reines, 1992 and Fig. 5, B and C), the nascent RNA in this experiment underwent only limited shortening. Hence, the size and half-life of cleavage products derived from a single RNA differed in the presence and absence of nucleotides (Figs. 2 and 3, this work and Reines, 1992). The brief half-life of cleaved RNAs seen here most likely resulted from their rapid re-extension in the presence of nucleotides, implying that transcript shortening preceded SII activation of elongation. Furthermore, these data prove that transcript cleavage is neither an artifact of nucleotide depletion nor of elongation complex immunoprecipitation.

The accumulation of runoff transcripts after 15 min suggested that the cleaved RNAs served as precursor to full-length RNAs (Fig. 2). To better resolve chain cleavage from chain elongation, we sought an approach that would allow us to more readily detect the products of RNA chain extension through site Ia. Thus, we studied SII-activated readthrough in the presence of a limited set of nucleotides. Immunoprecipitated elongation complexes were washed free of unincorporated nucleotides to reduce the NTP concentrations to less than 50 nM each (Reines, 1992). Washed complexes were then supplied with SII and 800 μM each of GTP, CTP, and UTP. The 3' termini of RNAs ending at site Ia map to 4 consecutive T residues on the non-transcribed DNA strand (Reines et al., 1987) as indicated in Fig. 3A. The template sequence down-
The presence of SI1 and all four nucleotides. Six reaction equivalents of washed elongation complexes were incubated with bovine brain SII (12 µg) and 800 µM each of ATP, GTP, CTP, and UTP. After 20, 45, 65, 95, and 900 s at 28 °C, one reaction equivalent was removed, and RNA was isolated for electrophoresis. Two shortened intermediates are indicated by an asterisk. The identity of the other RNAs are as indicated in the legend to Fig. 1B.

Stream from site Ia is such that in the presence of SII, UTP, GTP, and CTP transcript Ia (=205 nucleotides) can be extended by only 13–16 nucleotides (Fig. 3A); thus, elongation through site Ia should be readily observed. The kinetics of elongation under these conditions clearly demonstrated that nascent RNA was first cleaved and only subsequently elongated past site Ia (Fig. 3B). The prolonged half-life of these cleaved RNAs was probably due to a requirement for the insertion of an A residue into the shortened RNA between the cleavage product terminus (see legend to Fig. 3A) and site Ia (Fig. 3A). Apparently, enough residual ATP was present in the reaction to support this insertion. Alternatively, this elongation could represent the incorporation of a non-cognate nucleotide into RNA. In any case, the simplest interpretation is that transcript cleavage preceded and could be resolved from RNA extension during SII-activated transcription elongation.

These data suggested that RNA chains elongated by RNA polymerase II in an SII-dependent manner must pass through an intermediate that was shorter than the original transcript. This was demonstrated by an experiment using the chain-terminating nucleotide 3'-O-methyl-GTP. Washed elongation complexes were permitted to elongate their RNA chains in the presence of SII, 3'-O-methyl-GTP, UTP, and CTP (Fig. 3C, lane 3). If SII allowed the direct extension of transcript Ia without going through a shortened intermediate, transcript Ia would be extended by 12–15 nucleotides before the chain-terminating analog was inserted (Fig. 3A). If, however, chain shortening was an obligatory intermediate in the SII stimulation reaction, re-extension of the cleavage product would be prevented since after cleavage the chain-terminating G-residue would be inserted before the transcript could be significantly elongated. This would result in a limited extension product shorter than transcript Ia. Indeed, after 30 min at 28 °C only shortened transcripts were observed (Fig. 3C). This was consistent with the idea that incorporation of 3'-O-methyl-GMP into the transcript prevented the shortened RNA from serving as a precursor for chain elongation through site Ia. We emphasize that this reaction contained elongation factor SII and all nucleotides necessary for direct extension of transcript Ia. Thus, it appears that RNA chain shortening is a prerequisite to transcription through site Ia since preventing the extension of the cleaved RNA did not reveal an alternative means of elongating RNA chains, for example via the direct extension of Ia-RNA.

**Fig. 2.** Time course of transcription through site Ia in the presence of SII and all four nucleotides. Six reaction equivalents of washed elongation complexes were incubated with bovine brain SII (12 µg) and 800 µM each of ATP, GTP, CTP, and UTP. After 20, 45, 65, 95, and 900 s at 28 °C, one reaction equivalent was removed, and RNA was isolated for electrophoresis. Two shortened intermediates are indicated by an asterisk. The identity of the other RNAs are as indicated in the legend to Fig. 1B.

**Fig. 3.** Transcript shortening precedes SII-mediated transcription elongation. Panel A, RNA sequence around site Ia within the first intron of the human histone gene. The position of the 3'-ends formed when RNA polymerase II stops transcription are underlined and are collectively referred to as site Ia (Reines et al., 1987). The first A residue downstream of site Ia is boxed (□). The tentative site of cleavage which generates the major product (Reines, 1992) is indicated by an arrow. Panel B, transcription through site Ia after the addition of SII, GTP, UTP, and CTP. Washed elongation complexes were incubated with bovine brain SII and 800 µM each of UTP, CTP, and GTP. After 0, 0.5, 1, 4, 8, and 15 min at 28 °C, aliquots were withdrawn and labeled RNA was isolated for electrophoresis. The RNA that extends to the first A residue downstream from site Ia is indicated (□). The migration position of other RNAs are as indicated in the legend to Fig. 1B. Panel C, transcription through site Ia in the presence of 3'-O-methyl-GTP. Washed elongation complexes were incubated with 800 µM each of UTP and CTP, bovine brain SII, and either 800 µM GTP (lane 2) or 830 µM 3'-O-methyl-GTP (lane 3), for 30 min at 28 °C. RNA was isolated and analyzed by electrophoresis and autoradiography. The migration position of transcript Ia, run in an adjacent lane, is shown (Ia). Other RNAs are identified as described in the legend to Fig. 3B.
SII-dependent Chain Shortening Is Not Pyrophosphorolysis—Pyrophosphorolysis, reversal of the nucleic acid biosynthetic reaction, can be carried out by DNA polymerases (Deutscher and Kornberg, 1969; Atkinson et al., 1969) and bacterial and bacteriophage RNA polymerases (Maitra and Hurwitz, 1967; Krakow and Fronk, 1969; Kassavetis et al., 1986; Arndt and Chamberlin, 1990) in the presence of millimolar levels of pyrophosphate (PPi). Since RNA shortening by RNA polymerase II was dependent upon added SII, it seemed unlikely that cleavage resulted from pyrophosphorolysis (Reines, 1992). Nevertheless, it was important to rule out the possibility that SII acted as a PPi generator and to show that SII-dependent RNA shortening was distinct from pyrophosphorolysis. After 30 min in the presence of PPi and MgCl2, RNA polymerase II elongation complexes shortened a small but significant fraction of RNA chains (Fig. 4A, lane 3). Since the 32P label was present at the 5'-end of these RNA chains, nucleotide residues were removed from their 3'-ends. This was an SII-independent, PPi-dependent process that yielded a product distinct from that seen in the SII-dependent, PPi-independent cleavage of nascent RNA chains (Fig. 4A, compare lanes 1 and 3). The simplest interpretation was that this reaction represented pyrophosphorolysis. Previous work has shown that no significant RNA cleavage took place when washed complexes were incubated with MgCl2, in the absence of both PPi and SII (Reines, 1992).

![Image](image-url)

**Fig. 4.** RNA polymerase II elongation complexes can carry out pyrophosphorolysis. Panel A, pyrophosphate-dependent transcript shortening. Washed elongation complexes were incubated for 30 min at 28 °C with 7 mM MgCl2 and bovine brain SII (2 μg), or 1.8 mM sodium pyrophosphate as indicated above the figure. RNA was isolated and analyzed by electrophoresis and autoradiography. Panel B, effect of pyrophosphatase on transcript cleavage. Washed elongation complexes were mixed with 7 mM MgCl2 and either bovine brain SII (2 μg, lanes 1 and 2) or 1.5 mM sodium pyrophosphate (lanes 3 and 4) in the presence (+) or absence (−) of 10 units of yeast inorganic pyrophosphatase. (One unit of inorganic pyrophosphatase liberates 1.0 μmol of inorganic orthophosphate/min.) The reactions were then incubated at 28 °C for 30 min before RNA was isolated for electrophoresis. Three pyrophosphatase-sensitive RNAs are indicated by asterisks.

To demonstrate further that we were observing two distinct processes, pyrophosphorolysis and SII-dependent transcript cleavage, we inhibited pyrophosphorolysis by the addition of pyrophosphatase. Pyrophosphatase hydrolyzes inorganic PPi, to orthophosphate and thus, prevents pyrophosphorolysis (Cooperman, 1982; Tabor and Richardson, 1990). Washed elongation complexes were allowed to undergo SII-dependent or PPi-dependent RNA shortening in the presence of pyrophosphatase (Fig. 4B). Whereas the extent of PPi-dependent transcript shortening was dramatically reduced in the presence of inorganic pyrophosphatase (Fig. 4B, lanes 3 and 4), SII-dependent RNA cleavage was unaffected by the enzyme (lanes 1 and 2). We take this as evidence that washed RNA polymerase II elongation complexes can undergo pyrophosphorolysis and that it is chemically distinct from the more efficient and extensive RNA cleavage activated by SII.

**SII Can Activate the RNA Cleavage Reaction in Other RNA Polymerase II Elongation Complexes—**To this point we have observed nascent RNA cleavage in only two elongation complexes, the prominent one arrested at site Ia and a minor, less well-characterized site downstream (+325) in this template (pAdTerm-2, Reines, 1992 and Figs. 2 and 3). We tested the ability of an additional elongation complex to cleave its nascent RNA in response to SII. Washed elongation complexes containing RNA polymerase arrested at site Ia were allowed to extend their transcripts in the presence of added SII, UTP, GTP, and CTP as described above (Fig. 3). The majority of polymerase molecules became arrested at a downstream location where the first A residue was required. Small amounts of readthrough were seen which result in the extension of this RNA by two nucleotides to the next required A residue. These complexes were washed by precipitation and resuspension with anti-RNA antibody and challenged with rat liver SII for varying periods of time (Fig. 5A). By 10 min, chain shortening was apparent (Fig. 5A, lane 6). Thus, an SII-activated RNA-cleavage activity seems to be a general trait of an RNA polymerase II elongation complex. (Rat liver SII was used here since relatively large amounts of highly purified protein can be obtained in a concentrated form during the isolation of rat liver initiation factors. Thus far, purified bovine, human, and rat SII appear functionally indistinguishable.)

In some experiments run-off RNAs appeared shortened. This raised the possibility that some RNA polymerase molecules remained in functional elongation complexes for a significant time after they had reached the end of a duplex DNA template. If so, it would provide an opportunity to study RNA cleavage in more elongation complexes arrested at a site distinct from Ia. Like the complexes shown in Fig. 5A, these elongation complexes also represent RNA polymerases that were arrested for a reason other than their dependence upon SII for transcription elongation. Labeled runoff RNA was synthesized in vitro from two different linear templates (Fig. 5, B and C) and subjected to immunoprecipitation with anti-RNA monoclonal antibody. The precipitate was washed free of nucleotides and incubated with rat liver SII for varying times. Indeed "runoff" RNAs became progressively shorter with increasing incubation times. A small fraction of the RNAs were shortened by as many as 100 nucleotides judging by their mobility versus marker RNAs of known size (Fig. 5B). Since the addition of nucleotides resulted in efficient transcript extension (Fig. 5, B and C, chase lanes), cleaved RNA remained in functional elongation complexes. Only a portion of the runoff RNA, which was resolved as a closely spaced doublet (Fig. 5C), was capable of being shortened, perhaps indicating that only a fraction of the RNA remained associated with the template and RNA polymerase II in an
**Fig. 5.** Other elongation complexes possess SII-activated nascent transcript cleavage activities. Panel A, an RNA polymerase II elongation complex arrested downstream of site Ia can cleave its nascent RNA. Elongation complexes arrested at site Ia were assembled and washed (lane 1) as described under “Materials and Methods.” Washed complexes were incubated at 28 °C with SII (lane 2) or SII and UTP, CTP, and GTP for 20 min (lanes 3–6) as described in the legend to Fig. 3B. These “ATP-starved” complexes (lanes 3–6) were washed by immunoprecipitation and incubated for 0 (lane 3), 0.5 (lane 4), 2 (lane 5), or 10 min (lane 6) at 28 °C with approximately 5 × 10^4 absorbance units (280 nm) of rat liver SII (TSK-phenyl 5-PW fraction). Samples were prepared for electrophoresis and autoradiography as described under “Materials and Methods.” Panels B and C, runoff transcript cleavage. The plasmids pAdTerm-2 (B) or pDNAdML (C) were linearized with NdeI and transcribed in vitro with rat liver RNA polymerase II and general initiation factors. Reactions containing runoff RNAs (530 nt and 250 nt) pulse-labeled with [α-32P]CMP were subjected to immunoprecipitation with anti-RNA monoclonal antibody. Precipitates were washed and incubated with [α-32P]CMP in a manner that is inhibited, perhaps allosterically, by α-amanitin.

RNA polymerase II elongation complexes. The most important observation presented here is that nascent RNA cleavage precedes, and appears to be an obligatory intermediate in, SII-mediated transcriptional readthrough of a specific transcription arrest site. The association of nascent RNA cleavage with SII-regulated transcription elongation strongly suggests that the nuclease is causally involved in the readthrough process. The available data, including α-amanitin experiments, suggest that RNA polymerase II harbors the catalytic residues involved in the nuclease activity. This is not surprising since DNA polymerases, reverse transcriptases, RNA polymerase I (Huet et al., 1976), and bacterial RNA polymerase (Surratt et al., 1991) possess intrinsic nuclease activities. It has recently been reported that amino acids in the catalytic center of barnase, a bacterial ribonuclease, are shared with two other bacterial ribonucleases and the second largest subunits of Drosophila and yeast RNA polymerase II (Shirai and Go, 1991). Alternatively, SII may contain all or part of the catalytic site. If so it must interact with RNA polymerase II in a manner that is inhibited, perhaps allosterically, by α-amanitin.

The transcription arrest site studied here lies in a bent region of DNA (Kerppola and Kane, 1990). Evidence suggests that such a DNA helix represents a population in which bent isomers exist in equilibrium with relatively unbound or linear isomers (Koo et al., 1986). A model of SII-assisted readthrough at this site which accommodates this feature of the template has been put forth (Reines, 1992). It states that RNA polymerase II arrested at the site Ia bend prevents isomerization of bent DNA to an elongation-permissive, unbent form. SII, acting as an allosteric ligand, binds the elongation complex and activates a latent nuclease in RNA polymerase II. As a result of shortening the 3′-end of the growing RNA chain, polymerase moves relative to the template allowing renewed interconversion between bent and unbent DNA, followed by the resumption of transcript elongation. The findings presented here fulfill a prediction of this model: namely, that nascent RNA cleavage precedes SII-dependent transcript elongation. Our experiments with the chain terminator, 3′-O-methyl-GTP show that even in the presence of SII, and all nucleotides necessary for direct transcription from site Ia, elongation does not take place (Fig. 3C). Instead, we observe a new transcription intermediate: an RNA polymerase II elongation complex that bears a cleaved nascent transcript. Although it is possible that RNA cleavage may not accompany readthrough at all SII-dependent arrest sites, it is likely that the short half-life of the cleavage product seen in the presence of nucleotides probably precluded detection of this event in previous studies of SII-mediated readthrough (Reinberg and Roeder, 1987; Rappaport et al., 1987; SivaRaman et al., 1990; Bengal et al., 1991).

Elongation complexes located at template positions other than site Ia were also responsive to SII-activated RNA cleavage. It should be noted that the relative propensity for nascent transcript cleavage differs markedly between complexes. In washed elongation complexes, cleaved RNAs derived from transcript Ia were observed seconds after exposure to SII (Reines, 1992) whereas other complexes cleaved their RNAs slowly requiring many minutes or hours to obtain significant cleavage (Fig. 5). These findings show that a DNA bend is not required for SII activation and suggests that in vivo SII could stimulate transcription past other kinds of impediments to elongation. These could include nucleosomes or other DNA-bound proteins. If RNA polymerase "retreats" from a blockage site through RNA shortening, it may be able to

elongation complex. However, a significant number of RNA polymerase II molecules that synthesized runoff RNA did not appear to dissociate from these DNA termini. Collectively, the data of Fig. 5 show that many different RNA polymerase II elongation complexes possess the ability to cleave their nascent RNA chains in response to elongation factor SII. Therefore, elongation complexes other than those stopped at either SII-dependent transcription arrest sites or sites coincident with a bend in the template can perform this function.

**Discussion**

We have examined the relationship between SII-activated nascent transcript cleavage and transcription elongation by RNA polymerase II elongation complexes. The most important observation presented here is that nascent RNA cleavage precedes, and appears to be an obligatory intermediate in, SII-mediated transcriptional readthrough of a specific transcription arrest site. The association of nascent RNA cleavage with SII-regulated transcription elongation strongly suggests that the nuclease is causally involved in the readthrough process. The available data, including α-amanitin experiments, suggest that RNA polymerase II harbors the catalytic residues involved in the nuclease activity. This is not surprising since DNA polymerases, reverse transcriptases, RNA polymerase I (Huet et al., 1976), and bacterial RNA polymerase (Surratt et al., 1991) possess intrinsic nuclease activities. It has recently been reported that amino acids in the catalytic center of barnase, a bacterial ribonuclease, are shared with two other bacterial ribonucleases and the second largest subunits of Drosophila and yeast RNA polymerase II (Shirai and Go, 1991). Alternatively, SII may contain all or part of the catalytic site. If so it must interact with RNA polymerase II in a manner that is inhibited, perhaps allosterically, by α-amanitin.

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sample repeatedly regions of DNA which are only readthrough permissive part of the time, thereby increasing the probability of efficient transcript elongation. This mechanism may explain how the extremely stable RNA polymerase II elongation complex can be assisted in finishing gene transcription through numerous different types of barriers to transcript elongation.

To our knowledge this is the first demonstration of pyrophosphorolysis catalyzed by a specific eukaryotic transcription complex. Furthermore, SII-activated RNA cleavage by pyrophosphatase. Thus, SII does not appear to activate the phorolysis. Accordingly, we think that SII-activated RNA cleavage does not require PPi addition and is insensitive to the complex is distinct from pyrophosphorolysis in that the strand transfer function of yeast DSTa/SII may facilitate DNA strand transfer activity has shown that it is highly homologous, if not identical, to elongation factor SI1 (Clark et al., 1991; Kipling and Kearsey, 1991). This is consistent with our finding that SI1 defective in cleavage is hydrolytic. The process is initiated presumably when SI1 associates with the arrested elongation complex. This is consistent with our finding that SI1 defective in cleavage reaction by serving as a source of PPI for pyrophosphatase.

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